Metallomics: A Multidisciplinary Approach for the Qualitative and Quantitative Determination of Metalloproteins used as Health and Disease Markers

There have been estimations that over 30% of known proteins require metal cofactors for proper functionality. Since their discovery, metalloproteins have been the focus of research in biology and medicine because of their various functions in connection with protein folding and neurodegenerative diseases, metalloenzyme activity, and detoxification through transport and storage mechanisms. For example, quantitative determination of Zn, Cu, and Fe in brain proteins is of pressing interest for studying diseases like Alzheimer's and Parkinson's. This emerging science of "metallomics" requires versatile measurement tools allowing for trace detection, identification, and quantification of covalently bound metals in proteins or metal-protein complexes.

W.C. Davis, S.J. Christopher, S.E. Long, M. Vergne, A. Peck (Div. 839), L. Kilpatrick (Div. 838), and J. Baatz (Medical U. of SC)

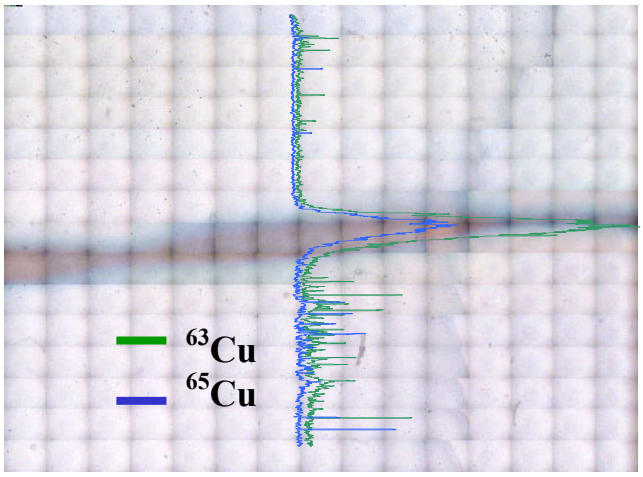
Protein isolation and determination of amount of substance, in addition to the chemical structural determination of a metalloprotein, is a challenging proteomics task that requires the use of numerous complementary biological processing and instrumental components. This biochemical problem dictates development of multidisciplinary analytical methodologies that contribute to solving the larger analytical problem. Simply put, it is difficult to quantify and identify a metalloprotein in a complex biological matrix with a single analytical technique. Protein separation by multidimensional liquid chromatography and/or 2D gel electrophoresis interfaced to inductively coupled plasma mass spectrometry (ICP-MS) may provide for rapid, metal-selective screening of target proteins, their subsequent quantification at low concentration levels, and finally, structure elucidation with the assistance of biomolecular mass spectrometry. It is here in concert with the aforementioned instrumental techniques where novel ICP-MS methods need to be developed for rapid screening and sensitive quantification of separated metalloproteins.

NIST researchers are working to combine multidimensional separation techniques with mass spectrometry to provide metal-selective screening, quantification, and structural information of target proteins.

Here we are beginning to test the utility of laser ablation (LA) ICP-MS for the multi-elemental screening of separated proteins on polyacrylamide gels. The analysis is relatively rapid, allowing mapping of whole gels in less than two hours; is amenable to multi-element data collection including the physiologically important elements P and S; and affords detection limits for important trace elements like Cu and Zn at the nanogram of protein level. This technique can be useful in identifying areas of elemental localization as a precursor to more in-depth studies using traditional organic mass spectrometry.

To date our research has concentrated on development of methods for separation of proteins, development of drying techniques for polyacrylamide gels to make them amenable for analysis by laser ablation ICPMS, and assessment of metal response as a function of protein concentration. A series of proof-of-principle experiments have been conducted.

Figure 1 shows a map of a protein band separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and an overlay of transient signals for ^{63}Cu and ^{65}Cu isotopes from laser ablation across a protein band separated by SDS-PAGE.



The trench created by the laser is approximately 110 μm in diameter. The width of a dried protein band is approximately 6 mm, therefore one ablation transect ablates approximately 1.8% of the lane containing the protein band.

The representativeness of the metal response remains an active area of research as initial studies indicate that the signal varies across a protein band. This may be an indica-

tion of metal inhomogeneity in the band, or the signal may fluctuate due to subtle changes in the gel matrix. We are in the process of identifying appropriate methods to address this through the use of isotopic signatures, employment of internal standards, and, if necessary, ablating (integrating) across the entire band. We will also test the response of proteins electroblotted onto PVDF membranes. These membranes offer the possibility of using F as an internal standard and should help to preconcentrate proteins and provide a more uniform, laser ablation-amenable matrix.

Impact: Development of qualitative and quantitative tools for separation and detection of metalloproteins will help benchmark measurements in biological systems and produce new classes of quantifiable biomarkers impacting health and environmental research.

Future Plans: **More fundamental research is planned to lay the groundwork for future quantitative measurements. For gels, preliminary research shows that external calibration using metalloprotein standards and LA-ICPMS may be possible, but a new strategy will be developed to quantify proteins separated on 2D gels through immersion into isotopic metal tracer solutions to investigate tracer uptake as a possible means of quantification with in-situ LA using the method of isotope dilution. A new strategy for the quantification of target metalloproteins from liquid samples is currently underway that will utilize size-exclusion chromatography with isotopic tracer internal standards mixed into the sample stream**.